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Distribution and origin of chromosomal races in the freshwater planarian *Dugesia polychroa* (Turbellaria: Tricladida)

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We present a karyotypic survey of the European freshwater planarian *Dugesia polychroa*, detailing frequencies of diploid and polyploid forms from 35 localities in seven countries. In this hermaphroditic species, diploids reproduce sexually and polyploids by pseudogamous parthenogenesis. Previous laboratory studies have shown that the two reproductive modes can interbreed, which may lead to new tri-, tetra-, and pentaploid lineages. We found four pure sexual, 25 pure parthenogenetic, and six mixed populations. Although some polyploid populations consisted entirely of triploids, most contained triploid and tetraploid individuals. Pentaploids were rare and reported for the first time from the field. In most populations, the higher ploidy levels were represented by fewer individuals. Our results indicate that occasional fertilisation of parthenogenetic eggs leading to genome addition is responsible for the maintainance of polyploid forms in natural populations of *D. polychroa*.

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In animal species with two separate sexes (gonochorists), the origin of parthenogenetic lineages is usually a rare event, and new lineages are genetically isolated from their progenitor species. Although theory predicts that parthenogenetic lineages cannot persist very long (GABRIEL et al. 1993), recent molecular studies have shown that such lineages can be very old, e.g., up to 5 million years in the salamander *Ambystoma* (SPOLSKY et al. 1992; HEDGES et al. 1992). The situation is different for parthenogenetic lineages that arise from hermaphroditic species as these can continue to produce viable sperm (BENAZZI-LENTATI 1970; CHRISTENSEN 1980). As a consequence, gene flow from parthenogens to their sexual progenitors is possible, and this could lead to frequent origin of new parthenogenetic lineages.

All planarians (Turbellaria) are hermaphroditic, and parthenogenesis is widespread in some freshwater species (Tricladida, Palludicola). BENAZZI and BENAZZI LENTATI (1976) have shown that parthenogens produce fertile sperm and, at least in some species, can hybridize with their sexual conspecifics. They also showed that hybridization can result in new parthenogenetic lineages in laboratory crosses. A variety of lineages with higher ploidy levels were obtained in subsequent generations. In order to assess the evolutionary significance of these results, it is necessary to know how frequently hybridization between reproductive modes occurs in nature. Collecting data on the geographic distribution and possible coexistence of sexuals and parthenogens is a first step towards this goal. In a mixed population, consisting of sexuals and parthenogens, one would expect to find a variety of polyploid forms as a result of hybridization between the two reproductive modes.

Many pure asexual populations are a mixture of different parthenogenetic lineages. This has been observed in parthenogenetic populations of various plant and animal taxa (SUOMALAINEN et al. 1987; DAWLEY and BOGART 1989; ASKER and JERLING 1992; MOGIE 1992). Genetic differences between lineages have been ascribed to mutational divergence, independent origin from sexuals, or occasional sexual processes in parthenogenetic populations. Such a sexual process may be occasional fertilisation of parthenogenetic eggs (genome addition) in our study species, the hermaphroditic planarian *Dugesia polychroa*. Though this process has not yet been found in natural populations of *D. polychroa*, it has been observed in parthenogenetic lineages that were generated in the laboratory, as well as in some te-

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traploid strains of the related species *D. benazii* (BENAZZI and BENAZZI LENTATI 1992).

The karyology and modes of reproduction of planarians have been described extensively by BENAZZI and BENAZZI LENTATI (1976). *D. polychroa* can reproduce both sexually and by pseudogamous parthenogenesis (sperm only activates parthenogenetic development of the egg but does not contribute genetically). Fissioning is a third mode of reproduction in planarians, but is typically absent in this species (BENAZZI et al. 1970; BRONDSTED 1969). The different reproductive modes are genetically fixed and considered as different chromosomal races or biotypes (BENAZZI 1957). Polyploidy is widespread among planarians, and typically associated with pseudogamic parthenogenetic reproduction or fissioning (BENAZZI and BENAZZI LENTATI 1976).

D. polychroa occurs in at least four chromosomal races termed biotypes A–D by BENAZZI (1957, 1963). Biotype A is diploid and sexual ($n = 4$, $2x = 8$). Biotypes B, C and D are polyploid and pseudogamous parthenogenetic. Biotype B is triploid ($n = 4$, $3x = 12$) and has synaptic oogenesis, i.e., triploid eggs are produced by meiosis after endoduplication. Biotype C is triploid ($3x = 12$) and oogenesis is asynaptic, i.e., triploid eggs are produced by mitosis. Biotype D is tetraploid ($4x = 16$) and asynaptic, i.e., tetraploid eggs are produced by mitosis. Biotypes A–D are genetically closely related and can interbreed (BENAZZI 1957).

The karyotypic distributions of most freshwater flatworms are known only fragmentarily in Western Europe. The most intensive study on *D. polychroa* is by BENAZZI (1957), who examined karyotypes from localities in Great Britain, Denmark, Germany, France, Switzerland, Austria and Italy. Additional data are available from the vicinity of Pisa, Italy (CANOVAI et al. 1985; CANOVAI and GALLENI 1988; CANOVAI 1989), Northern Ireland (MAGAGNINI 1962), Great Britain (REYNOLDSON and BELLAMY 1970), southern Sweden (MELANDER 1963), The Netherlands (VAN DER VELDE and DE VRIES 1985), France (DUTRILLEUX and LENICQUE 1971), Spain (GOURBAULT 1981), and Greece (BALL 1979).

The pattern arising from the previous studies is that sexuals are restricted to areas south of the Alps, with the exception of some populations in southern Sweden, whereas parthenogens are present all over Western Europe. This pattern agrees with many other studies on the geographic distribution of parthenogenetic organisms (LYNCH 1984; SUOMALAINEN et al. 1987), which have shown that parthenogens usually occur at higher latitudes, and can have a much wider distribution than their sexual relatives. The aim of the present study is to confirm the absence of sexual *D. polychroa* in central Europe, and to detail the distribution of different polyploid forms. Unlike previous studies, we report frequencies of different chromosomal races for each locality. The possibility that new polyploid lineages arise through fertilisation of unreduced eggs (genome addition) will be discussed.

Material and methods

D. polychroa inhabits ponds, lakes and quiet reaches of lowland streams (DEN HARTOG 1962; REYNOLDSON 1978). We collected worms between January 1993 and January 1995 by flushing them gently from the under-sides of stones picked from the water. Some sampling localities were chosen because the species had previously been reported to occur there, but many localities had not been investigated before. Data from localities that were visited more than once were pooled. The number of collected animals per locality ranged from one to several hundreds.

Karyotypes were established from regenerated blastemas or whole animals according to a modified protocol of REDI et al. (1982). After colchicine treatment (0.15 % for 2–4 h) tissue was hypotonized in distilled water for 15 min and fixed in an ice-cold mixture of 3 vol methanol and 1 vol acetic acid. Tissue was then cut into small pieces in a drop of 45 % acetic acid on an object glass and squashed under a cover glass. Slides were examined under phase-contrast (400x–1000x) on an Olympus BHS or BH2-RFCA microscope. Karyotype nomenclature is according to LEVAN and MÜNTZING (1963).

Results

We karyotyped 735 individuals from 35 localities in seven countries (Table 1 and Fig. 1). These include Great-Britain (1), The Netherlands (6), Belgium (1), France (2), Germany (12), Austria (1) and northern Italy (12). Ploidy levels ranged from diploid to pentaploid (Fig. 2a–d). Our data confirm the widespread distribution of *D. polychroa* in western Europe. Sexuals were only found

Table 1. Karyotypes and distribution of *Dugesia polychroa*. Locality numbers correspond to Fig. 1. For each locality, the total number of karyotyped individuals and the frequency of each karyotype are given

Locality	Number	Karyotype			
		2x = 8	3x = 12	4x = 16	5x = 20
Great Britain					
1. Sheffield	5	—	0.80	0.20	—
The Netherlands					
2. Abcoudermeer, Abcoude	21	—	0.86	0.14	—
3. 't Gein, Abcoude	7	—	1.00	—	—
4a. De Vecht, Uitermeer	24	—	0.75	0.25	—
4b. De Vecht, Hinderdam	7	—	0.57	0.43	—
5. Spiegelplas, Hinderdam	22	—	0.91	0.09	—
6. Hilversums Kanaal, Kortenhoef	6	—	0.83	0.17	—
Belgium					
7. Kempens Kanaal, Turnhout	2	—	1.00	—	—
France					
8. Nantua	29	—	0.17	0.79	0.04
9. Gardon	25	—	1.00	—	—
Germany					
10. Main, Zellingen	5	—	0.60	0.40	—
11. Main, Hallstadt	6	—	1.00	—	—
12. Regnitz, Pettstadt	6	0.67	0.33	—	
13. Regnitz, Hüttendorf	8	—	0.87	0.13	—
14. Altmühl, Treuchtlingen	10	—	0.80	0.20	—
15. Ammersee, Herrsching	24	—	1.00	—	—
16. Maisinger See, Maising	7	—	1.00	—	—
17. Würm, Starnberg	2	—	1.00	—	—
18. Starnberger See, Feldafing	2	—	1.00	—	—
19. Riegsee, Murnau	12	—	1.00	—	—
20. Chiemsee, Chieming	42	—	0.95	0.05	—
21. Waginger See, Tettenhausen	5	—	1.00	—	—
Austria					
22. Donau, Wien	3	—	1.00	—	—
Italy					
23. Lago di Como, Santa Marina	8	—	1.00	—	—
24. Lago di Garlate, Olginate	18	—	0.83	0.17	—
25. Lago di Toblino	17	1.00	—	—	—
26a. Lago di Caldonazzo west	129	0.73	0.25	0.02	—
26b. Lago di Caldonazzo southwest	70	0.71	0.21	0.06	0.02
26c. Lago di Caldonazzo east	52	1.00	—	—	—
27. Lago di Levico, Levico	20	1.00	—	—	—
28. Pontetetto, Pisa	5	0.80	—	0.20	—
29. Massa Pisana, Pisa	29	0.86	—	0.10	0.04
30. San Lorenzo a Vàccoli, Pisa	84	0.74	—	0.23	0.03
31. Borgo, Pisa	15	1.00	—	—	—
32. La Fattoria, Pisa	8	0.63	0.12	0.25	—

in Italy in 3 lakes in the Trentino region and at 6 localities around Mount Pisani near Pisa. These observations correspond with previous reports from these localities by BENAZZI (1957), CANOVAI et al. (1985), CANOVAI and GALLENI (1988) and CANOVAI (1989), except that Benazzi reports only parthenogens (biotype B) from Lago di Caldonazzo. In Lago di Toblino, Lago di Caldonazzo (one site), Lago di Levico, and Borgo near Pisa only sexuals were observed. At the other six localities where sexuals were found, they coexisted with polyploid pseudogamous parthenogenetic individuals. Interestingly, in Lago di Caldonazzo, the sample from the east shore consisted of sexuals only (N = 52), whereas the other two localities contained di-, tri, tetra-, and pentaploids ('west' N = 129 and 'southwest' N = 70). A manuscript detailing the microdistribution of different ploidy forms in Lago di Caldonazzo will be presented elsewhere (WEINZIERL and BEUKEBOOM, in prep.).

Triploids were found in all seven countries surveyed (Table 1 and Fig. 1). We generally did not

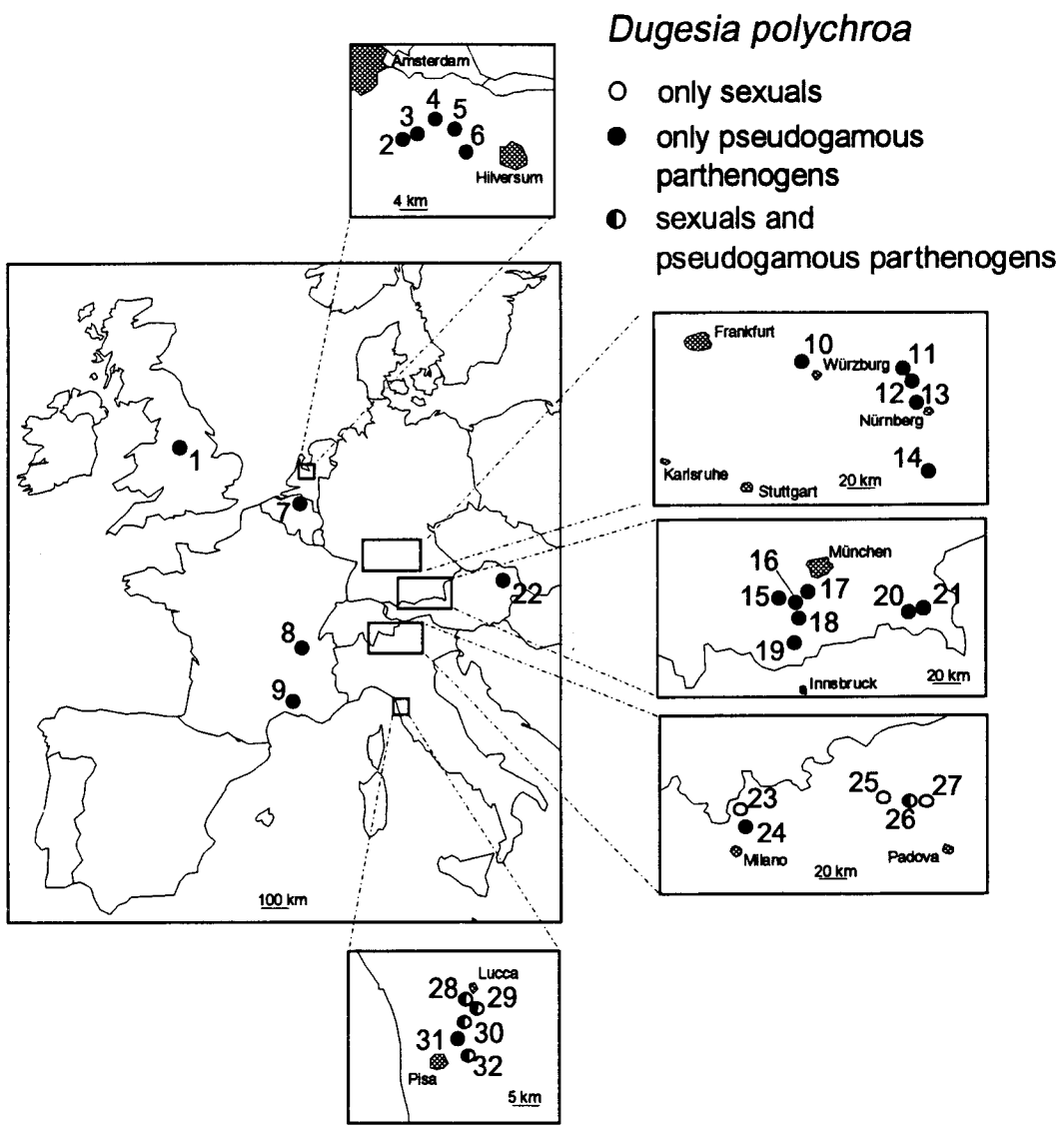

Fig. 1. Localities in Western Europe from which *Dugesia polychroa* has been collected.

determine whether these belonged to biotype B (synaptic female meiosis) or C (asynaptic). In the few cases where we observed female meiosis in triploids (one animal from the Ammersee, one from Lago di Garlate, and three from Lago di Caldonazzo), it was synaptic. This is consistent with BENAZZI (1957), who reports biotype B as the most widespread. Tetraploids were found at several localities in the Netherlands, Germany and Italy, and in one locality in Great Britain and France, respectively. We are not certain whether these belong to Benazzi's biotype D, or whether they are synaptic polyploids. We occasionally found pentaploids (three localities in Italy and one in France; Table 1). Pentaploids have not been reported from natural populations before. Fig. 3 shows that polyploid populations typically consisted of individuals with different ploidy levels and that the higher ploidy levels were represented by fewer individuals.

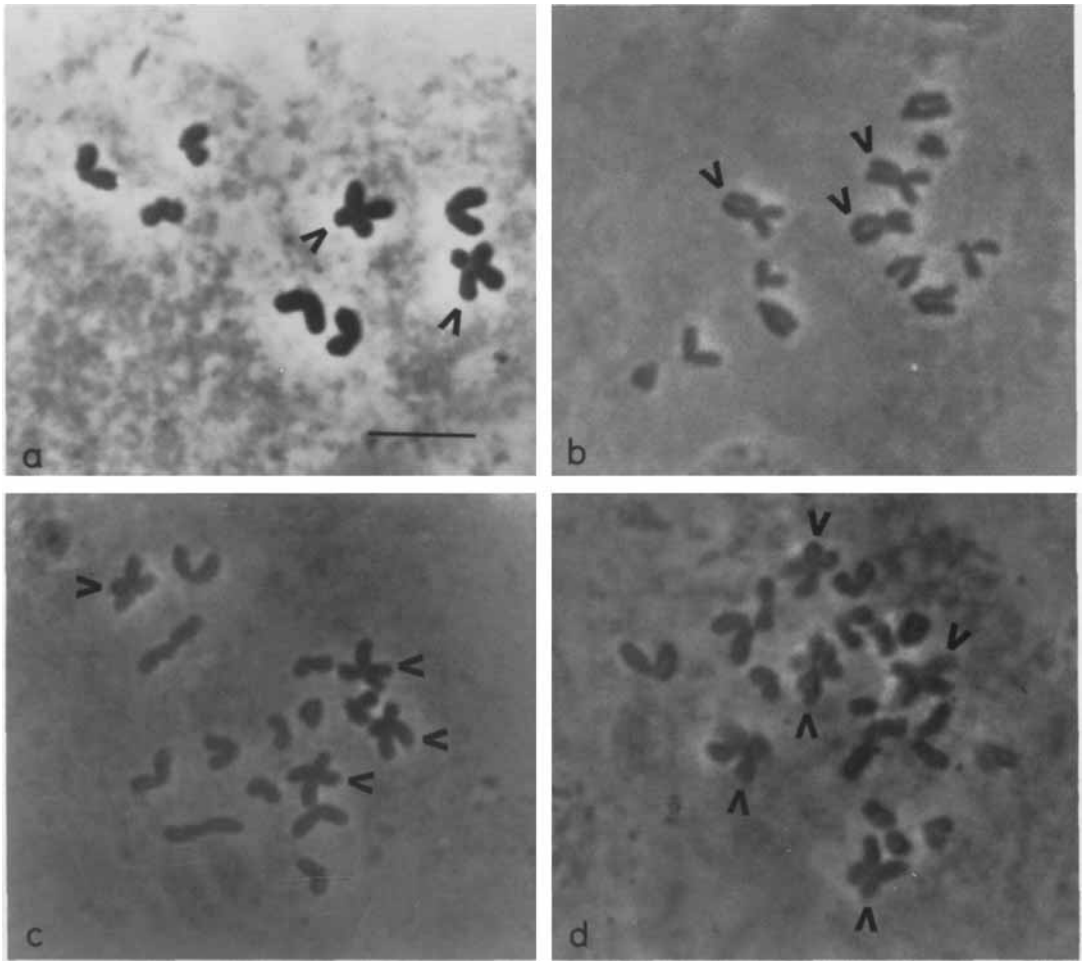

Fig. 2a–d. Diploid 2n = 2x = 8 (**a**), triploid 2n = 3x = 12 (**b**), tetraploid 2n = 4x = 16 (**c**), and pentaploid 2n = 5x = 20 (**d**) karyotype of *Dugesia polychroa*. Arrows indicate the large metacentric chromosomes. Bar = 5 μm.

Discussion

Geographic distribution

Our data confirm previous reports (BENAZZI 1957) that sexual *D. polychroa* (biotype A) are mainly restricted to southern Europe and apparently absent from central Europe. These results are in agreement with the general biogeographical rule that sexuals occur at lower latitudes than their parthenogenetic relatives (e.g., LYNCH 1984; SUOMALAINEN et al. 1987). They also show that the Alps form a borderline for sexuals, which are abundant in northern Italy, but absent in southern Germany. Because the Alps constitute a climatic border as well as a dispersal barrier, it is unclear whether this pattern is due to innate habitat preferences, or to colonization history after the latest glaciation, as known for other species (reviewed in SUOMALAINEN et al. 1987). Assuming REYNOLDSON (1966) is correct in that *D. polychroa* is very slow in colonizing new areas, the present distribution may simply reflect a better colonizing ability of parthenogenetic strains. Sexual populations which originate from a single fertilized individual may go extinct because of inbreeding depression, whereas this may not be the case for parthenogenetic populations.

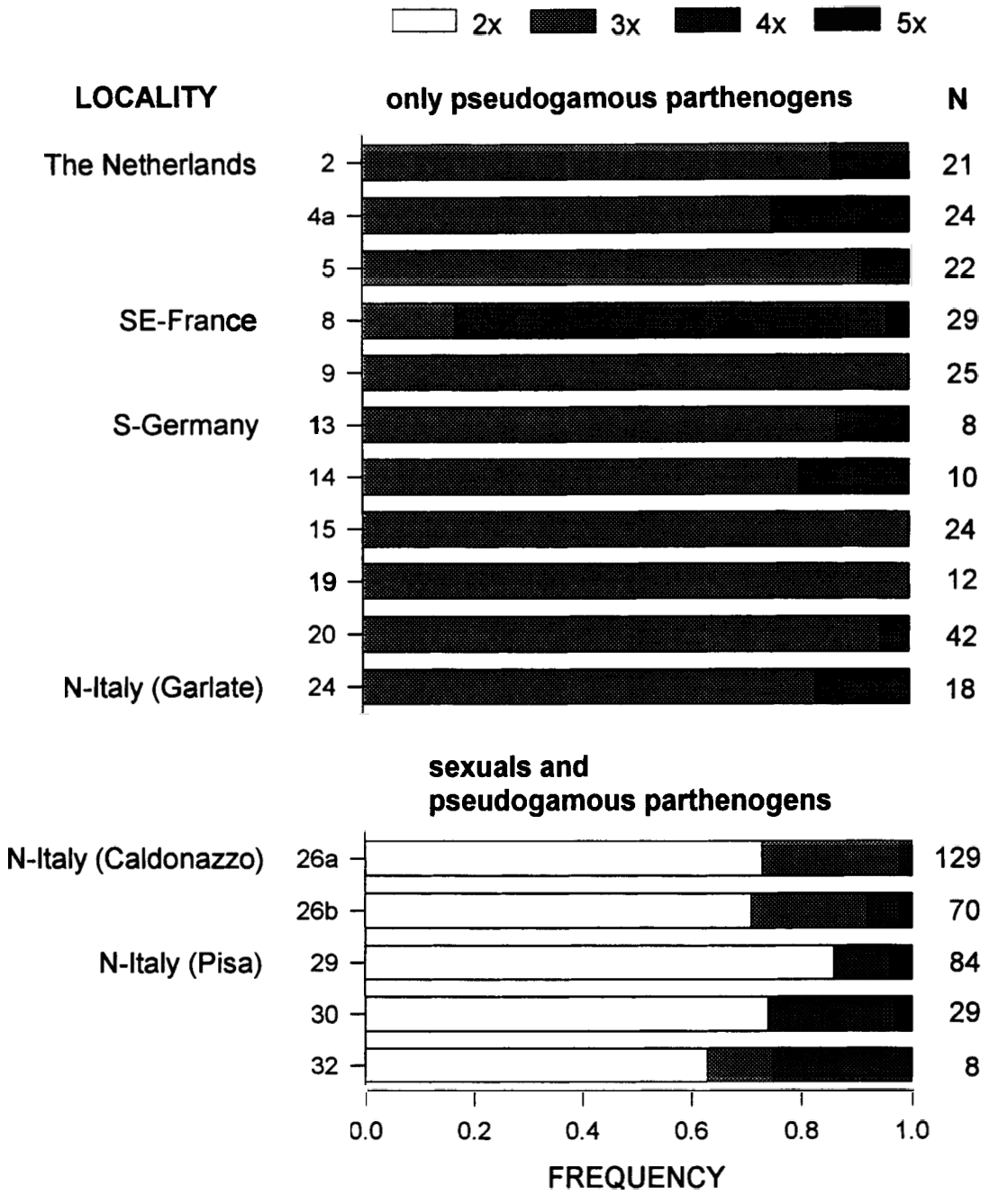

Fig. 3. Relative frequencies of chromosomal races of *Dugesia polychroa*. Only localities from which at least 8 individuals have been karyotyped are shown and localities with only sexuals are omitted. Population numbers refer to Table 1 and Fig. 1. N = number of individuals.

There are some inconsistencies with the explanations above. The sexual sibling species *D. lugubris* had obviously no problems colonizing central and northern Europe (BENAZZI and BENAZZI LENTATI 1976). Moreover, MELANDER's (1963) report of sexual *D. polychroa* from southern Sweden shows

that they can exist in a cold climate. These sexuals may have survived the latest glaciation in glacial refugia or may have originated from populations in southern Europe by long distance dispersal. Such dispersal may be aided by human activities, as evidenced by the recent immigration of *D. polychroa* onto the North American continent (BALL 1969; BODDINGTON and METTRICK 1974). Human activities are also believed to be responsible for the rapid spread during this century of the introduced North-American *D. tigrina*, which is now present all over Europe (BENAZZI 1993; RIBAS et al. 1989).

A more subtle trend is revealed by the relative abundances of different polyploid biotypes in purely polyploid populations (Table 1 and Fig. 3). Tetraploids occur at consistently higher frequencies in the Netherlands (localities 2–6) and central Germany (localities 10–14), than in southern Germany (localities 15–21). Of the two French populations, one consists mainly of tetraploids whereas the other is purely triploid. On the other hand, the area of Pisa (localities 28–32) is almost completely lacking triploids, which is consistent with CANOVAI (1989). The local coexistence of different ploidy levels could be explained by assuming that they use slightly different niches of the same habitat. However, the general pattern of rareness of individuals with high ploidy levels across localities is hard to explain with habitat selection only. We suggest that cytological mechanisms offer an alternative explanation.

Origin of polyploid forms

Our data show that populations of *D. polychroa* can consist of animals with different ploidy levels and that sexuals and pseudogamous parthenogens can coexist. An essential point is to consider how such a variety of biotypes can arise and persist in this flatworm species. We consider two processes to be acting on the origin of polyploid forms: (1) production of unreduced gametes by sexuals, and (2) occasional fertilisation of eggs in pseudogamous parthenogens.

As in many other organisms, polyploid lineages in flatworms are believed to have arisen from diploid sexuals (HANSEN-MELANDER et al. 1954; BENAZZI and BENAZZI LENTATI 1976). This may occur by occasional production of diploid eggs or sperm, which appears to occur in many organisms (TURGEON and HEBERT 1994) or by dispermy (two sperms fertilizing one egg). Although fertilization of unreduced gametes will immediately lead to new triploid individuals, the triploid state can only be maintained over generations when parthenogenetic reproduction is acquired together with triploidy. BENAZZI (1963) and BENAZZI and BENAZZI LENTATI (1976) have demonstrated that parthenogenesis in triploid *D. polychroa* is no immediate consequence of polyploidy, and that it is regulated by several genes. Indeed, the genetic basis for parthenogenetic and sexual reproduction appears to be polygenic in many species (LYNCH 1984), which suggests that certain genes which are responsible for pseudogamous parthenogenesis are already present in diploid sexuals. One then has to assume that these genes increase in number and/or expression in parthenogenetic lineages (BENAZZI LENTATI 1966).

Once parthenogenesis has evolved, new lineages may arise frequently after hybridisation between sexuals and parthenogens. In *D. polychroa* haploid sperm from polyploid parthenogens can fertilize haploid eggs of sexuals. Such hybrids have been frequently obtained in the laboratory (e.g., BENAZZI and BENAZZI LENTATI 1976; WEINZIERL unpublished). They usually behave like normal sexuals, but some individuals produce unreduced eggs (BENAZZI LENTATI 1970). This again leads to triploid individuals that sometimes reproduce by pseudogamous parthenogenesis (BENAZZI LENTATI 1966). Thus, in mixed populations, gene flow from pseudogamous parthenogens to sexuals may promote the repeated origin of new parthenogenetic lineages.

The new triploid lineages obtained by BENAZZI LENTATI (1966) produced unreduced eggs, but were not perfectly pseudogamous parthenogenetic. This means that quite frequently the sperm chromosomes were incorporated into the egg, thereby giving rise to tetraploid individuals. In this way, Benazzi Lentati was able to add one haploid set each generation. However, from pentaploids onwards, developmental abnormalities and spontaneous reduction in ploidy level started to occur. This could explain the absence of individuals with hexaploid or higher chromosome complements in natural populations.

We found mixed diploid-polyploid populations in Lago di Caldonazzo (localities 26a,b) and near Pisa (localities 28–32). Data from Lago di Caldonazzo show a polyploid series of tri-, tetra-, and pentaploids in sharply declining numbers (Fig. 3), which is consistent with the notion that the two higher ploidy levels arise recurrently by the addition of a haploid set, but numbers are kept low by

natural selection. The question remains whether triploids themselves arise frequently out of the sexual subpopulation, which is expected as a consequence of hybridisation between diploid and polyploid biotypes. The data from Pisa (localities 28–32) indicate that this may not be the case. Consistent with CANOVAI's (1989) results, tetraploids (25 individuals) were the most abundant polyploid form in the Pisa samples, whereas pentaploids (4 individuals) and triploids (1 individual) were rare (Fig. 3). The rareness of triploids indicates that the origin of new lineages in the Pisa populations occurs either very infrequently, or that newly arisen triploids do not persist in the population because of low fitness.

In Benazzi and Benazzi Lentati's experiments, polyploid series of tri-, tetra-, and pentaploids arose only from laboratory generated triploids, which were obtained after hybridisation between diploid sexuals and triploid parthenogens. Our data indicate that occasional fertilisation of unreduced eggs can occur within established triploid or tetraploid lineages, independently of hybridisation with diploids. In purely polyploid populations, individuals with the highest ploidy level were typically rare (see Fig. 3). The highest ploidy level was the least abundant in all 13 populations which contained more than one polyploid form, but no diploids. Consistent rarity of higher ploidy levels makes it unlikely that they are maintained because they occupy different ecological niches. Instead, it suggests that tetraploids originate recurrently from triploids, and pentaploids from tetraploids. Genome addition through failure of sperm expulsion has also been found in polyploid salamanders of the genus *Ambystoma* (ELINSON et al. 1992) and it is considered as the most likely origin of polyploid unisexual fish (VRIJENHOEK 1994). Recently, TURGEON and HEBERT (1994) reported evidence for genome addition from sexuals to parthenogens in ostracods.

The magnitude of the above mentioned processes acting on the evolution of parthenogenetic biotypes in *D. polychroa* remains unknown. Using genetic markers, we are currently investigating the amount of genetic variation among biotypes in different populations. We want to find out to what extent crosses between chromosomal races occur in mixed populations of *D. polychroa*, and how often these crosses lead to the origin of new polyploid lineages.

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